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Journal of Nutritional Biochemistry 17 (2006) 242–249
Journal of Nutritional Biochemistry

Effects of the type of dietary fat on acetylcholine-evoked amylase secretion and calcium mobilization in isolated rat pancreatic acinar cells ☆

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Received 5 April 2005; received in revised form 21 April 2005; accepted 28 April 2005

Abstract

Olive oil is a major component of the Mediterranean diet, and its role in human health is being actively debated. This study aimed to clarify the mechanism of pancreatic adaptation to dietary fat. For this purpose, we examined whether dietary-induced modification of pancreatic membranes affects acinar cell function in response to the secretagogue acetylcholine (ACh). Weaning male Wistar rats were assigned to one of two experimental groups and fed for 8 weeks with a commercial chow (C) or a semisynthetic diet containing virgin olive oil as dietary fat (OO). The fatty acid composition of pancreatic plasma membranes was determined by gas–liquid chromatography. For assessment of secretory function, viable acini were incubated with ACh and amylase of supernatant was further assayed with a substrate reagent. Changes in cytosolic Ca^{2+} concentration in response to ACh were measured by fura-2 AM fluorimetry. Compared to C rats, pancreatic cell membranes of OO rats had a higher level of monounsaturated fatty acids and a lower level of both saturated and polyunsaturated fatty acids, thus, reflecting the type of dietary fat given. Net amylase secretion in response to ACh was greatly enhanced after OO feeding, although this was not paralleled by enhancement of ACh-evoked Ca^{2+} peak increases. In conclusion, chronic intake of diets that differ in the fat type influences not only the fatty acid composition of rat pancreatic membranes but also the responsiveness of acinar cells to ACh. This mechanism may be, at least in part, responsible for the adaptation of the exocrine pancreas to the type of fat available.

Keywords: Dietary fat; Olive oil; Pancreatic acinar cells; Membrane fatty acids; Cell calcium; Rat

1. Introduction

It is generally accepted that biological membranes do not have a constant composition. Some factors, such as age, physiologic state, cell type, antioxidant capacity, metabolic activity or the diet, can modulate the structure and function of cell membranes. Previous observations [1] from the authors' research group in Spain, along with those by other researchers [2,3], indicate that dietary fat composition influences the fatty acid profile of phospholipids in both cellular and subcellular membranes of different organs. These effects can be quite fast. In the newly weaned rat pups, diets containing 20% (w/w) fat from different sources affected the fatty acid composition of intestinal brush border membranes after only 40 h of feeding [4], and the effects of coconut, olive or sunflower oil diets (10% w/w) were evident in liver microsomes [5] after 6 days. A period of 6 weeks has been found to be enough to induce changes in adipocyte plasma membrane of weanling rats [6] and some reports [7,8] indicate that the rat kidney, lung and erythrocytes are also responsive to modifications in dietary fatty acids during early postnatal life (4–6 weeks after weaning). Differences in fatty acid profile seem to be permanent provided the animals are kept on the same dietary

Abbreviations: ACh, acetylcholine; CCK-8, cholecystokinin-octapeptide; PSS, physiological salt solution; BSA, bovine serum albumin; LDH, lactate dehydrogenase; PMCA, plasma membrane calcium ATPase; SERCA, sarcoplasmic/endoplasmic reticulum calcium ATPase; IP3, inositol 1,4, 5-trisphosphate.

☆ This study was funded by the Spanish Ministry of Science and Technology (grant no. BFI2002-02772). Personal support of M.D. Yago, M.A. Martínez and N. Audi was provided by the Junta de Andalucía.

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doi:10.1016/j.jnutbio.2005.04.009

treatment [4], but the effects can be changed back to the original situation by refeeding the former diet [9]. It should be noted that the extent or rate of diet-induced changes depends on the tissue. Erythrocyte and liver membranes have been shown to have a marked adaptive capacity as demonstrated in the rat, rabbit and swine [1,7,10,11]. For other tissues such as the brain, skeletal muscle and heart, a great variability has been reported in their sensitivity to dietary changes [1,7,8,12,13], which probably reflects the influence of the antecedent diet [11], age, percentage and type of dietary fat and duration of the feeding period.

Changes in membrane composition have been shown to influence cell function [2,14,15]. Proposed mechanisms include alteration of the activity of membrane-associated enzymes, modification of hormone binding or responsiveness, and changes in the regulation of gene expression [2]. We have confirmed that the type of dietary fat strongly influences the fatty acid composition of rat [16] and rabbit [17] pancreatic membranes. In the rat study, this was accompanied by a change in the secretory activity and mobilization of intracellular Ca^{2+} stimulated by cholecystokinin-octapeptide (CCK-8) in viable pancreatic acini [16]. Interestingly, by using the method of direct cannulation of the pancreatic duct in anaesthetized rats, we have been able to find a modification of CCK-induced secretory output as a function of the type of fat previously fed [18], which indicates that the modulating effect of this nutrient on exocrine pancreatic function is not limited to the cellular level.

Another pathway that contributes to the regulation of exocrine pancreatic secretion is the cholinergic one. Vagal stimulation evokes in anaesthetized rats a very similar response to that obtained with CCK-8 [19]. The effects of both acetylcholine (ACh) and CCK-8 involve Ca^{2+} signaling but Ca^{2+} mobilization is triggered by different means [20].

This study was launched to determine whether, similar to our previous observations with CCK-8, dietary-induced modification of pancreatic membranes affects acinar cell function and signaling in response to ACh. Confirmation that dietary fat composition can change the responsiveness of the pancreas to different secretagogues would help to clarify the mechanism of pancreatic adaptation to dietary fat. For this purpose, we have examined ACh-stimulated amylase secretion and intracellular Ca^{2+} mobilization in viable pancreatic acini from rats fed for 8 weeks with a virgin olive oil diet or a commercial chow. Olive oil was chosen because of its preferential use in our geographical area. Moreover, this fat source is a major component of the Mediterranean diet and its role in human health is currently debated.

2. Materials and methods

2.1. Animals and diets

All procedures were approved by the ethical committees of the University of Granada and the Spanish Ministry of Science and Technology. Animals were handled according to the guidelines of the Spanish Society for Laboratory Animal Sciences and killed humanely. Seventy male weaning Wistar rats weighing 40–55 g and supplied by the Animal Farm at the University of Granada were divided into two groups (35 each) so that the average weight per group was the same. Rats of the olive oil group (OO) were fed for 8 weeks with a semipurified diet that was essentially the AIN-93G diet [21] except that (i) total fat content was increased from 70 to 100 g/kg at the expense of carbohydrate and (ii) the fat source was virgin olive oil (Fedeoliva, Jaén, Spain). The control group (C) was fed over the same 8-week period with a standard laboratory chow for this species (Panlab A04, Panlab Laboratories, Barcelona, Spain).

The fatty acid composition of the diets (Table 1) was determined by gas–liquid chromatography as described later for the membrane fractions. The OO diet was prepared at the Nutrition Unit of the Animal Farm (University of Granada, Spain), vacuum-packed in plastic bags, sealed and sent to our laboratory, where it was stored at 4°C in the dark. During the 8-week feeding period the animals were housed individually in a temperature-controlled room (22±1°C), kept on a 12-h light–dark cycle and given free access to water and food. Body weight was recorded weekly. Food intake was determined daily in a subset of rats from both groups ($n=8$ each) by recording weigh-back that was discarded. All rats in the present study were killed by severance of the vertebral column after an overnight fast (food was always withheld from 8:00 to 8:30 p.m. and the time of killing was 9:00 to 9:30 in the next morning).

2.2. Isolation and analysis of cell membranes

The rat pancreatic plasma membrane fractions were prepared from gland homogenates by differential and sucrose gradient centrifugation [22]. Next, the technique of Lepage and Roy [23] was used, a method that combines lipid extraction and fatty acid methylation in a one-step reaction. A gas–liquid chromatography system, model HP 5890 series II (Hewlett Packard, Palo Alto, CA, USA),

Table 1
Fatty acid content of the experimental diets

	Commercial rat chow	Virgin olive oil diet
C16:0	15.45	9.81
C16:1n-7	1.16	0.63
C18:0	4.15	3.96
C18:1n-9	21.59	76.75
C18:2n-6	45.67	6.81
C18:3n-3	3.98	0.66
SFA	19.6	14.02
MUFA	22.75	77.67
PUFA	49.65	8.31

Results are expressed as percentages of total fatty acid content (mean values of four replicates).

equipped with an automatic injector and a flame ionization detector, was used to analyze fatty acids as methyl esters. Chromatography was performed using a 60-m-long capillary column (32 mm internal diameter and 20 mm thickness) impregnated with Sp 2330 FS (Supelco, Bellefonte, CA, USA).

2.3. Preparation of pancreatic acini

A suspension of viable single cells and small acini was prepared from rat pancreas by previously described methods [16]. Briefly, the pancreas was rapidly removed and trimmed free of fat and nodes in a physiological salt solution (PSS, pH 7.45) that comprised (per liter): bovine serum albumin (BSA), 2 g; soya bean trypsin inhibitor, 1 g; glutamine, 0.3 g; vitamin mixture (BME vitamins solution 100×; Sigma, St. Louis, MO, USA), 10 ml; amino acid mixture (BME amino acids solution 50×; Sigma), 25 ml. The PSS also contained (mM): NaCl, 120; KCl, 7.2; sodium pyruvate, 6; sodium fumarate, 7.1; sodium glutamate, 6; glucose, 14; KH_2PO_4, 2; $MgCl_2$, 1.2; HEPES, 24; $CaCl_2$, 2. Next, 2 ml PSS containing 44 U purified collagenase per milliliter (type CLSPA; Worthington Biochemical, Lakewood, NJ, USA) was injected into the pancreas, which was subsequently digested at 37°C for 30 min in a shaking water bath, washing with fresh collagenase solution every 10 min. This was followed by vigorous manual agitation. The dispersed acini were placed in cold PSS containing 40 g BSA per liter and centrifuged (50×*g*, 4 min, 4°C). The pellet was then resuspended in PSS containing 2 g BSA per liter. Following gentle pipetting, the digested material was filtered and centrifuged (50×*g*, 4 min, 4°C). Cell viability was, at different times, monitored by trypan blue exclusion. All experiments were conducted after a 30-min equilibration period at 37°C in PSS.

2.4. Amylase release

Amylase release was measured as described previously [24]. In brief, acini were suspended in incubation medium (PSS containing 10 g BSA per liter and 0.5 mM $CaCl_2$). Samples (500 ml) were then incubated at 37°C for 30 min with 0.1, 1 or 10 μM ACh (acetylcholine chloride, Sigma), and this was followed by centrifugation at 350×*g* for 2 min (4°C). Acini exposed to the incubation medium alone served as unstimulated controls (basal release). Amylase activity in supernatant fractions was determined with the Phadebas reagent (Pharmacia & Upjohn, Barcelona, Spain) and expressed as a percentage of total amylase content at the beginning that was released into the extracellular medium during the incubation.

2.5. Measurement of cytosolic free Ca^{2+} concentration

Acini were suspended in Na–HEPES solution (pH 7.40) containing (g/l) BSA, 2; soya bean trypsin inhibitor, 0.1, and (mM) $CaCl_2$, 1; NaCl, 130; KCl, 5; HEPES, 20; KH_2PO_4, 1.2; $MgSO_4$, 1; glucose, 10. Cells were loaded with 2 μM fura-2 AM (Molecular Probes Europe, Leiden, The Netherlands) for 20 min at 37°C in a shaking water bath, washed and resuspended in the same solution without BSA or trypsin inhibitor. For quantification of fluorescence, samples of acinar suspension (2 ml) were placed in a quartz cuvette in a Perkin-Elmer LS 50B spectrofluorimeter (Perkin-Elmer, Beaconfield, Bucks, UK) and continuously stirred at 37°C. Fura-2-loaded cells were excited at 340 and 380 nm, and emission was monitored at 510 nm. After baseline measurement, ACh was added into the cuvette to a final concentration of 0.1, 1 or 10 μM. At the end of each experiment, maximum and minimum fluorescence ratio values were obtained by the respective addition of digitonin (final concentration 80 mM) and EGTA (pH 8.7, EGTA final concentration was 7 mM). Values for cytosolic free Ca^{2+} concentration were calculated according to Grynkiewicz et al. [25].

2.6. Assessment of pancreatic injury

Histological studies of the pancreas were conducted. Glands from rats fed with the control or olive oil diet were carefully isolated, trimmed of fat and fixed in 10% neutral buffered formalin. After fixation, the tissues were dehydrated with ethanol and embedded in paraffin, sectioned at a thickness of 5–6 μm and stained with hematoxylin and eosin for microscopic examination.

In order to check that cell viability was good enough after the tissue dissociation protocol used to prepare the cell suspension and confirm that consumption of the experimental diets was not affecting this parameter, we measured lactate dehydrogenase (LDH) leakage as an indicator of membrane integrity. Thus, although the trypan blue test was qualitatively employed at different times during the digestion procedure, the LDH method would serve better for quantitative purposes. Leakage of the cytosolic enzyme LDH was determined in different batches of cells after the equilibration period (before starting the experiments). Briefly, aliquots of cell suspension were centrifuged (350×*g* for 2 min at 4°C) and LDH activity in supernatants was measured with a commercial kit (ref. TOX-7, Sigma) based on NAD^+ reduction. Results were expressed as a percentage of total enzyme activity measured in parallel samples after lysis of acini.

2.7. Calculations and statistical analysis

To calculate the integrated response for each Ca^{2+} experiment, basal Ca^{2+} concentration values before the addition of ACh were averaged to estimate the baseline and this was subtracted from each of the stimulation values. The numbers obtained in this way were then summed. The same number of data points was used for the calculation of all integrated areas. Time taken for this calculation was 237.5 s.

The rate of recovery to prestimulation values in the Ca^{2+} experiments was calculated by fitting single exponential [$y=y_o e^{(-Kt)}$] to the decay of Ca^{2+} response for 100 s after the spike, and the decay constant (K) was obtained for each

individual experiment. Unless otherwise stated, the results presented in the text, tables and figures are expressed as mean±S.E. Differences between the dietary groups were assessed by the independent-samples Student's *t* test using SPSS software (SPSS for Windows, version 12.0.1, 2003; SPSS, Chicago, IL, USA). Only values of $P<.05$ were considered significant.

3. Results

3.1. Food intake and rat weight

Initial values of body weight were similar in group OO (46.3±2.7 g, $n=35$) and group C (47.5±1.3 g, $n=35$). Feeding diets differing in the amount and type of fat did not affect final body weight, which was comparable in both experimental groups (OO: 343.4±12.4 g, $n=35$; C: 352.9±9.4 g, $n=35$). Food intake was significantly lower in OO animals than in C animals throughout the feeding period, although the caloric intake was similar (data not shown). There were no differences between the groups in pancreas weight (OO: 1.302±0.058 g, $n=35$; C: 1.418±0.074 g, $n=35$).

3.2. Fatty acid composition of pancreatic cell membranes

Intake of experimental diets profoundly influenced the fatty acid composition of pancreatic cell membranes (Table 2). Membranes of the OO group were characterized by significantly higher levels of total monounsaturated fatty acids (MUFA) and significantly lower levels of total saturated (SFA) and polyunsaturated fatty acids (PUFA) as compared with the C group.

3.3. Assessment of pancreatic injury

No histological alterations were detected in the pancreas of rats from both experimental groups (Fig. 1). Values for LDH leakage before the beginning of the experiments were low and no statistical differences were revealed among the experimental groups at the level of $P<.05$ (OO: 2.83±0.592%, $n=6$; C: 2.91±0.921%, $n=6$).

Table 2
Fatty acid composition of pancreatic cell membranes of rats fed with a commercial chow (C) or a virgin olive oil diet (OO) for 8 weeks

	C group	OO group
C16:0	35.94±0.83*	24.78±0.39
C18:0	9.85±0.80*	7.01±0.50
C18:1n-9	19.13±4.00*	41.75±2.01
C18:2n-6	12.07±1.83*	4.80±0.49
C20:4n-6	8.38±1.54	5.33±0.65
C18:3n-3	0.56±0.10	0.35±0.05
SFA	50.74±2.16*	38.87±2.10
MUFA	24.96±4.20*	49.01±2.77
PUFA	24.30±3.34*	13.07±1.34

Results are expressed as percentages of total fatty acid content. Values are means±S.E. for $n=12$.

* $P<.05$ compared with the OO group.

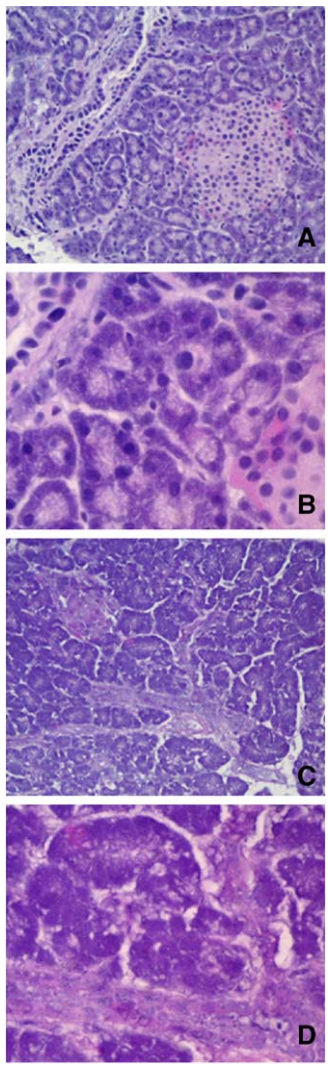

Fig. 1. Histology. Pancreas of rats fed with a standard chow (A, B) or a semisynthetic diet containing olive oil as the fat source (C, D) (original magnification 200× for A and C and 600× for B and D). Histological findings after hematoxylin–eosin stain were not different among the groups.

3.4. Amylase release

Basal values of amylase release were comparable in acini from OO rats (6.01±0.602%, $n=9$) and C rats (5.73±0.705%, $n=12$). In both groups, net amylase release (above basal) in response to 0.1–10 μM ACh showed the highest value at 1 μM (Fig. 2). Larger and smaller concentrations of ACh resulted in a decrease in amylase release. However, at any particular concentration of the secretagogue, feeding with the OO diet for 8 weeks was associated with greater values of ACh- stimulated net amylase release in pancreatic acini compared with the control (C) diet (Fig. 2).

3.5. Cytosolic free Ca^{2+} concentration

Basal values of cytosolic Ca^{2+} concentrations were significantly higher in OO rats (173.4±10.29 nM, $n=23$)

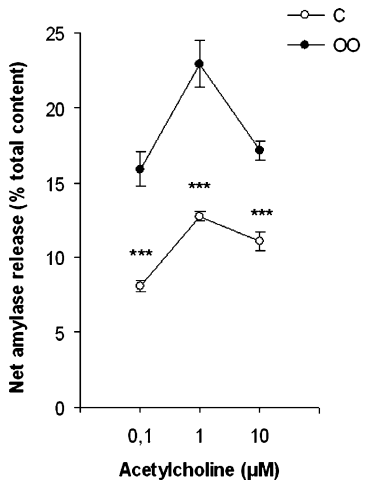

Fig. 2. Net amylase release (increase above basal) stimulated by acetylcholine in pancreatic acini isolated from rats fed with a standard chow (C) or a semisynthetic diet containing olive oil as the fat source (OO). Amylase released during the incubation with the secretagogue (30 min, 37°C) is expressed as a percentage of total initial content. Results are presented as means±S.E. of 10–30 separate experiments (in each experiment, values were determined in duplicate). Mean values were significantly different between the dietary groups; ***$P<.001$.

than in C rats (115.9±4.82 nM, $n=21$). The time-course changes in cytosolic free Ca^{2+} concentration evoked by ACh were similar in both groups and showed the typical responses to ACh in acinar cell suspensions, that is, a prompt increase followed by a slow decline toward a suprabasal level.

Data in Table 3 show that the Ca^{2+} response expressed as peak increases above basal after the addition of 0.1 and 10 μM ACh was significantly ($P<.05$) higher in cells from rats fed with the control diet (C) than in those isolated after OO feeding. Although this trend is observed for 1 μM ACh, the difference was less pronounced. Cytosolic Ca^{2+} concentration stimulated by 0.1 and 10 μM ACh was also significantly greater in C rats when expressed as integrated response (Table 3).

It can be observed in Fig. 3A that ACh stimulation in C rats apparently results in a faster recovery of Ca^{2+} concentration to the prestimulation level compared with OO rats. Calculation of the rate of recovery (see Materials and Methods and Fig. 3B) further confirmed this observation. The results showed that the decay constant (K) was significantly ($P<.05$) higher in C rats than in OO rats for 1 μM ACh (C: 0.0074±0.0007 s^{-1}, $n=6$; OO: 0.0048±0.0007 s^{-1}, $n=8$) and 10 μM ACh (C: 0.0127±0.0008 s^{-1}, $n=11$; OO: 0.0069±0.0009 s^{-1}, $n=9$).

Table 3
Effect of graded concentrations of ACh on cytosolic Ca^{2+} concentration in suspensions of pancreatic acinar cells isolated from rats fed with a commercial chow (C) or a virgin olive oil diet (OO) for 8 weeks

	C group ($n=4$–11)	OO group ($n=6$–9)
0.1-μM ACh		
Peak (nM)	271.5±12.5	252.9±31.6
Increase above basal (nM)[a]	134.4±15.4*	73.5±15.5
Integrated response (nM)[b]	5836±349*	2945±460
1-μM ACh		
Peak (nM)	388.2±21.9	416.9±40.6
Increase above basal (nM)	271.7±19.3	239.8±21.5
Integrated response (nM)	8400±455	11526±2170
10-μM ACh		
Peak (nM)	664.8±66.8*	420.4±27.9
Increase above basal (nM)	556.9±68.1*	254.2±30.1
Integrated response (nM)	11969±653*	7954±1420

Values are means±S.E.

[a] The increase above basal represents the difference between peak and basal value.

[b] Calculated as described in Materials and methods.

* $P<.05$ compared with the OO group.

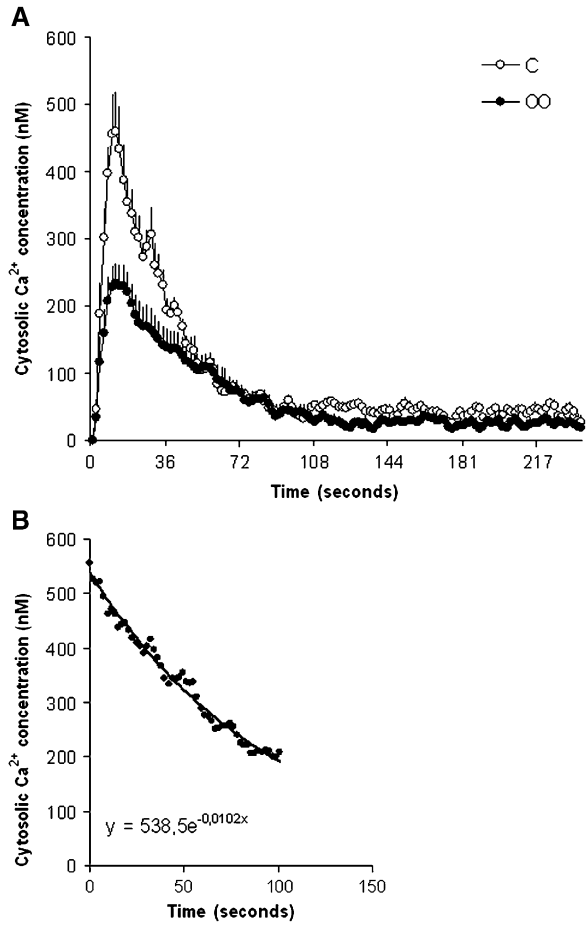

Fig. 3. Effect of ACh on cytosolic Ca^{2+} concentration in pancreatic acinar cells. (A) Ca^{2+} response to 10 μM ACh in cells from rats fed with a commercial chow (C) or an olive oil diet (OO) is expressed as increase above basal. For any particular experiment, the averaged basal value was subtracted from each of the stimulation ones. Time was normalized so that the time $t=0$ s corresponds to the last point before the ACh-induced rise. Results are means±S.E. of 11 (C) and 9 (OO) independent experiments. (B) An example of exponential decay curve fitted to the Ca^{2+} data obtained from an individual experiment in pancreatic acinar cells of a rat fed with the olive oil (OO) diet. Time was normalized so that the time $t=0$ s corresponds to the peak value after addition of 10 μM ACh. For details of calculation, see Materials and methods.

4. Discussion

Diet intake in OO rats was lower than in C rats. However, the amount of calories ingested per week was comparable in both groups. It is well known that rats have the ability to regulate their caloric intake by adjusting food consumption as a function of the caloric content of the diet [26]. As a logical consequence, feeding with diets differing in the amount and type of fat did not affect in this study the final body and pancreas weight. These results are consistent with those obtained by others [27] and indicate that this experimental model is not associated with the existence of morphological changes. Histological findings after hematoxylin–eosin stain of pancreatic sections did not differ among OO and C groups. Chronic intake of excessive amounts of dietary fat has been reported to induce deleterious effects on pancreatic morphology and physiology. In our experimental conditions, although the OO diet was richer in fat that the C diet, we could not find in OO pancreases the alterations (swelling and cytoplasmic vacuolization) described by others [28]. The adaptation to the diets was, from the above points of view, satisfactory.

That our dietary protocol was appropriate for our purposes was also confirmed by the membrane fatty acid analyses showing, like in previous works [16,17], the sensitivity of the pancreas to dietary fat changes. After 8 weeks on the diet, pancreatic cell membranes of C animals had a higher level of total SFA and PUFA, whereas a greater level of total MUFA was found in OO animals. The existence of dietary-induced changes in the fatty acid profile of rat pancreatic membranes was previously found to be associated with modifications in CCK-8-evoked secretory activity in anaesthetized rats [18] and in acinar cell function and signaling evoked by the same secretagogue [16]. Thus, the results of the membrane fatty acid composition obtained in the present study after feeding rats the OO and C diet support the use of this model to assess if there is a differential response to ACh.

Adaptation to the diets did not modify basal amylase release. Basal values in OO and C rats were similar between them and comparable to those described in the literature [29,30]. This contrasts with the results of basal Ca^{2+} concentration. Values in C group were in the range of those reported by others [30,31], whereas Ca^{2+} concentration in OO cells was markedly higher. Determination of LDH leakage in this study indicates good membrane integrity in both dietary groups. Therefore, it is possible that the differences in membrane composition are altering the mechanisms that maintain low resting levels of Ca^{2+} in pancreatic acinar cells. In unstimulated cells, cytosolic Ca^{2+} concentration is sustained by a balanced array of influx (favored by gradient), efflux and intracellular sequestration mechanisms, with calcium ATPases in plasma membrane (PMCA) and endoplasmic reticulum (SERCA) being responsible for pumping Ca^{2+} out of the cell and into intracellular stores [32], respectively. Our results suggest that the activity of PMCA and/or SERCA may be reduced in acinar cells from OO rats, leading not only to resting Ca^{2+} levels significantly elevated but also to a slower recovery rate of Ca^{2+} concentrations after ACh stimulation. Another possibility might be an increased Ca^{2+} leakage from the internal stores. Certainly, differences in Ca-ATPase activity of heart sarcolemma have been reported after feeding animals different types of fat [3]. Dietary-induced changes in the composition, and possibly structure, of membrane phospholipids may affect Ca-ATPase turnover [33].

The dose–response curve for ACh-induced amylase release in pancreatic acinar cells is typically bell-shaped, reaching a maximum at around 1 μM [29,34]. We also observed the strongest secretory effect at 1 μM ACh in both groups. Quantitatively, however, marked differences existed between them, since net amylase secretion in response to all concentrations of ACh was drastically enhanced after OO feeding. Surprisingly, ACh-evoked Ca^{2+} responses were smaller in OO cells than in C cells. With the present data we cannot ascertain whether the reduced ACh-induced Ca^{2+} mobilization in OO rats involves Ca^{2+} entry, Ca^{2+} release from stores, or both. It is well known that the initial rise in Ca^{2+} transients is mainly due to Ca^{2+} released from internal stores. Then, without discarding an effect on Ca^{2+} entry, our finding that the peak increases over basal are lower in cells from OO rats suggests that at least Ca^{2+} release from stores is likely affected. This might be due to a reduction in the filling state of ACh-releasable Ca^{2+} pools and/or a limitation in the production or effectiveness of the mediators that participate in the Ca^{2+} mobilization pathways. Experiments are in progress in order to investigate the nature of such differences. The fact that ACh-induced amylase secretion, but not the Ca^{2+} response, was enhanced in OO cells supports that other intracellular pathways different from Ca^{2+} signaling may mediate this distinct effect. Nevertheless, if Ca^{2+} had any role at all as the mediator, this would be exerted through the existence of a different temporal pattern, as shown by the significantly slower recovery rate of the Ca^{2+} transients in OO rats.

The differences in acinar Ca^{2+} mobilization and secretory activity in the present study are likely related to the dietary-induced changes in cell membrane composition. These changes have been shown to modify insulin binding in rat adipocytes and the activity of membrane-associated enzymes in liver [2]. Differential enrichment in certain fatty acids may influence the accessibility of the muscarinic receptor, the interaction with G proteins or the functionality of such enzymes as phospholipases and protein kinase C which are known to interact with cell membranes during their activation. Early reports indicated that cholinergic stimulation of pancreatic amylase secretion was exerted through M3 muscarinic receptors. In fact, the acinar cell was thought to lack an M1 muscarinic receptor. More recently, the results published in the literature [35] clearly demonstrate the existence of both subtypes on acinar cells. What is more, Schmid et al. [36] have shown

that carbachol-induced amylase secretion in isolated acinar cells was more potently inhibited by the M1-selective antagonist telenzepine than by M3-selective antagonists. In this respect, it is interesting to note that manipulation of membrane fatty acid composition by dietary means has been shown to affect the density of M1 receptors in hippocampal neurons [37]. Whether a similar mechanism is responsible for the differences in amylase secretion pattern among groups C and OO in this study deserves further investigation.

In addition to their structural role, membrane fatty acids participate themselves as mediators in signal transduction. Muscarinic receptors of pancreatic acinar cells are mainly linked to activation of phospholipase C, which leads to hydrolysis of phosphatidylinositol bisphosphate and subsequent production of inositol 1,4,5-trisphosphate (IP3) and diacylglycerol. The membrane modifications in the present study could reasonably involve an alteration in the phosphoinositide turnover and a change in the supply of inositol lipid precursors of IP3. A different production of IP3 in acini from rats fed with the OO or C diet may explain our results of Ca^{2+} response to ACh. On the other hand, diacylglycerol (possibly containing different acyl moieties as a consequence of the observed changes in the membrane) may have resulted in our study in differential activation of protein kinase C, a crucial modulator of the secretory machinery of acinar cells. This is strongly supported by the finding in guinea pig epidermis that diacylglycerol with an 18:2n-6 metabolite at the 2-position inhibited protein kinase C isozymes compared with 1,2-dioleylglycerol [38].

The lack of a positive correlation between Ca^{2+} increases above basal and amylase secretion contrasts with previous work in acinar cells from rats fed with different dietary fats where CCK-8-evoked amylase secretion correlated the CCK-8-stimulated Ca^{2+} responses [16]. Why this different behavior of CCK-8 and ACh? Both substances are potent Ca^{2+} releasers. However, CCK not only stimulates secretion of digestive enzymes, but also promotes pancreatic growth, whereas ACh mainly stimulates secretion. It has been suggested that CCK (hormone) and ACh (neurotransmitter) are linked to different specific combinations of messengers and therefore trigger specific Ca^{2+} signals and specific physiological responses. Thus, whereas both ACh- and CCK-elicited Ca^{2+} signaling are completely dependent on both IP3 and ryanodine receptors, the response induced by CCK (but not ACh) is also dependent on nicotinic acid adenine dinucleotide phosphate and cyclic ADP-ribose [20]. In addition, CCK (and not ACh) can activate phospholipase D [39].

In conclusion, the present data confirm that chronic intake of diets that differ in the type of fat added influences not only the fatty acid composition of rat pancreatic cell membranes but also the responsiveness of acinar cells to acetylcholine, as assessed by Ca^{2+} mobilization and amylase release. This represents a possible mechanism of adaptation of the exocrine pancreas to the type of fat available.

Acknowledgments

We are grateful to Dr. José María Martínez de Victoria (Pathology Service, Hospital de Jaén, Spain) for assistance in conducting the histological study and for his valuable comments.

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